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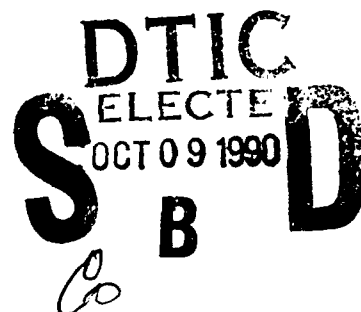
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<p>The objectives of this project, therefore are to evaluate (1) the emetic properties of radioprotectants of the WR2721 family in monkeys in relation to modifications of gastrointestinal motility; (2) the role of the area postrema in WR2721-induced vomiting and gastric suppression; and (3) the possibility of preventing the side effects of radioprotectants with antiemetics. During this period the investigators have continued to study the emetic effects of WR2721 in monkeys, and have measured the concurrent action of this radioprotectant on gastric motility and gastric emptying of liquids and solids. In addition, they have determined the role of the area postrema in WR2721-induced vomiting and gastric suppression in beagle dogs. Finally, they evaluated the possibility of preventing WR2721-induced vomiting with zacopride.</p>			
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FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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RADIOPROTECTANTS AND GASTRIC FUNCTION

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INTRODUCTION

Nausea and vomiting sometimes induced by WR2721 and other thiol derivative radioprotectants represent a serious drawback in their use in military personnel for at least two reasons. Firstly, the incapacitation induced by nausea and vomiting would preclude its prophylactic administration unless military personnel would not have to be operational. Secondly, in case of exposure to ionizing radiation subsequent to the administration of WR2721, one would not know if the presence of vomiting is due to the radioprotectant or to radiation.

The mechanism and treatment of these emetic side effects are not established, and the role of possible alterations of gastrointestinal motility is at present unknown. In dogs, the use of chronically implanted electrodes has demonstrated that nausea and vomiting induced by apomorphine are preceded and accompanied by relaxation of the stomach and retroperistaltic activity of the duodenum (Weisbrodt and Christensen, *Gastroenterology* 63:1004-1010, 1972). Using a primate model, we recently confirmed these observations, and demonstrated that radiation induced vomiting was accompanied by a slowing of gastric electrical control activity, slowing of gastric emptying, and suppression of acid output (Laporte et al: *Dig Dis Sci* 29:565A, 1984). Finally, we found that the anti-emetic domperidone effectively prevents radiation-induced vomiting in dogs

(Dubois et al: Gastroenterology 86:444-448, 1984), although this medication is not effective in monkeys (Dorval et al, Gastroenterol 1985; 89:374-380).

The objectives of this project, therefore, were twofold: (1) to determine the emetic properties of radioprotectants of the WR 2721 family in monkeys in relation to modifications of gastrointestinal motility; (2) to determine the role of the area postrema in WR2721-induced vomiting and gastric suppression; and (3) to evaluate the possibility of preventing the side effects of radioprotectants with antiemetics.

During the past 12 months, we have continued to study the emetic effects of WR2721 in monkeys, and have measured the concurrent action of this radioprotectant on gastric motility and gastric emptying of liquids and solids. In addition, we have determined the role of the area postrema in WR2721-induced vomiting and gastric suppression. Finally, because endogenous opiates suppress gastric emptying and are increased following radiation and stress, we also examined their possible role in this phenomenon.

METHODS

1. Evaluation of the emetic and gastroplegic effects of WR2721 and of the involvement of endogenous opioids (8 rhesus monkeys).

Eight monkeys weighing 2.5 to 6.8 kg were trained to sit quietly in a primate restraining chair, which allowed their study without the use of anesthetics. The animals were monitored using videotaping; and gastric motility and gastric emptying were determined as described below, before and after administration of WR2721 (37.5, 75,

and 150 mg/kg). WR2721 (BK45589, AP-10-205) was weighed and diluted in 10 ml of saline at room temperature within 10 min of the start of the administration. It was given as a slow intravenous infusion over a 10 min period using a Model 975 Harvard pump.

The monkeys were first habituated to sit in a primate restraining chair before the start of the actual experiments. During these three initial chairing sessions performed over a two- week period, the animal were continuously watched for the first 15 min and then at regular intervals for the subsequent 3 hr before being returned to their cage. On the morning of each habituation and, subsequently, during drug studies, they were chaired after an overnight fast, placed in a lighted, ventilated booth positioned in front of a gamma camera and studied during the subsequent 4 hours. They were then brought back to their cage and fed.

The plasma concentration of beta-endorphin was measured as previously described (Dorval et al, Gastroenterol 1985; 89:374-380), before and after intravenous and intragastric administration of WR2721, WR3689, and WR1065 (150 mg/kg). In addition, blockade of opiate receptors was performed using naloxone (0.004 mg/kg/min) given as a slow subcutaneous injection starting 30 min before the meal.

Two types of studies were performed concurrently to evaluate gastric function: (a) measurement of gastric motility with implanted electrodes; and (b) measurement of gastric emptying using radionuclide imaging.

A. Gastric motility.

Three weeks before the start of the experiments and after one week of habituation to the primate restraining as described above, 3 rhesus monkeys were anesthetized (induction with ketamine, 10 mg/kg, continued with halothane and nitrous mixture, 4%

at induction, 1% for maintenance) and operated using aseptic surgery. A mid-line laparotomy was performed, 5 pairs of silver/silver chloride electrodes were sutured on the stomach serosa, and 3 strain gauges were sutured onto the gastric serosa, providing a measurement of gastric electrical and mechanical activity. The teflon-coated wires connected to the electrodes and strain gauges were passed through the abdominal wall and through a subcutaneous tunnel created under the skin of the back of the animal, to reach a plug fixed onto the skull with dental cement. The principle of this surgical technique is similar to the one used in other animal species (Grunden and Linburn, *J Pharm Sci*, 1969; 58:1147-1148). Following surgery, the animals received bicillin (200,000 U/kg/day; IM), and were closely observed during one week. In our experience, this design has allowed chronic recording of gastrointestinal activity for up to 6 months. After recovery, the animals were again chaired and 4 electrocardiographic electrodes were placed on the skin of their abdomens, thus permitting the concurrent recording of the cutaneous electrogastrogram. The skull plug and skin electrodes were connected through a cable to a Beckman R612 multichannel recorder, thus permitting amplification of the biopotentials and their recording on paper. The amplified analog signals were simultaneously taped on an Ampex 2230 analog tape recorder. The taped signal was subsequently digitized, and analyzed on a computer using Fast Fourier transform and locally developed programs (Guetta et al, *Gastroenterology*, 1986, 91:183A). These evaluations were performed on a MNC/DECLAB-23 and the Wavetek 4-Channel Signal Analyzer. In previous studies, we observed that a good correlation existed between skin and gastrointestinal potential recordings in monkeys in the basal state.

B. Measurement of gastric emptying.

Gastric emptying was determined in monkeys chaired as described above, concurrently with the recording of gastric motility in three animals, and independently in the 5 other animals. Two different techniques were used.

i. Marker dilution technique

A previously described and validated marker dilution technique (Dubois et al, J Clin Invest 1977; 59:255-263) was used to determine gastric secretion and gastric emptying during a 40-min fasting period and for 60 min after the injection of an 80 ml water load (postload period). In the present studies, this technique was slightly modified in that ^{99m}Tc -DTPA (diethylene triamine pentaacetic acid) was used instead of phenol red as the marker as recently described (Dorval et al, 1985; 89:374-380). This intubation method requires only the sequential sampling of the gastric contents and permits concurrent measurement of intragastric volume, gastric emptying and gastric secretion. A 12 French double lumen nasogastric tube was placed in the stomach and its position was verified by the water recovery test. Starting 45 min later, samples of the mixed gastric contents were aspirated just before and immediately after intragastric administration of 5 to 20 ml of a ^{99m}Tc -DTPA test solution (0.03 mCi/100 ml H_2O ; pH 7.4; 37°C) and were centrifuged. The clear supernatant of each sample was assayed for Tc- 99m concentrations using an Ultrogamma autogamma counter (LKB Instr, Turku, Finland) and for titratable acidity using electrometric titration to pH 7.4 (Radiometer, Copenhagen, Denmark). These determinations were repeated every 10 min during the basal period and after intragastric instillation of an 80 ml water load containing Tc- 99m DTPA (0.003 mCi/100 ml; pH 7.4; 37°C).

Intragastric volumes of fluid (V1,V2...) and amounts of Tc-99m (Tc1,Tc2...) were determined at the time of each sampling using the dilution principle and locally developed computer programs. Fractional emptying rate (g) was then determined for each 10-min interval (t) between two dilutions, assuming that emptying was a first-order process (exponential) during a given 10-min interval and using equation:

$$g = - [\log_e (Tc2/Tc1)]/t$$

Since g is allowed to vary from interval to interval, no general assumption has to be made regarding emptying over the total duration of the experiment. Net fluid output (Rv) in ml/min was then determined for the corresponding interval, assuming that it remained constant over the given interval and using equation:

$$Rv = [V2 - V1 \cdot \exp(-gt)] \cdot g / [1 - \exp(-gt)]$$

Intragastric volumes of fluid and masses of Tc-99m were then recalculated, taking into account these first estimates of fractional emptying and fluid output, which were in turn recalculated. This iterative process was repeated until the improvement of the solution became less than 1% per iteration. Similar calculations were used to determine gastric acid secretion.

ii. Studies of gastric emptying of liquid and solids

On the day before each study, rhesus monkeys were fasted overnight with free access to water. On the next morning, the animals were chaired as described above, and a 10-French nasogastric tube was placed into the stomach. A mixed solid-liquid meal containing 0.6 mCi Technetium-99m-sulfur-colloid (solid phase, composed of 1.2g Tc-tagged chicken liver cooked in a microwave oven and ground to particles of 0.5 to 1.0 mm in diameter) and 0.1 mCi Indium-111-DTPA (liquid phase) in 50 ml of water mixed

with the liver was prepared. In vivo tagging of chicken liver allows homogeneous and stable labeling of solid particles (Meyer et al, Amer J Dig Dis 1976; 21:293-304).

The mixed solid-liquid meal was injected directly into the stomach through a nasogastric tube after the chaired and intubated monkey was positioned in front of a 12-inch gamma camera interfaced with a computer. Radionuclide imaging was performed at 10-min intervals for three hours after this injection, thus permitting the determination of the amount of Tc-99m and In-111 present in the stomach and in the intestine. One-min imaging were taken from the front and from the back of the animal, thus permitting a calculation of the geometric mean of the amounts of radioactivity measured in these 2 pictures. The percentage of solids (Tc-99m) and liquids (In-111) remaining in the stomach at each time interval was calculated as the ratio of the stomach contents divided by the sum of the gastric and intestinal contents.

In order to test the hypothesis that the poor oral bioavailability of WR2721 is related to the fact that oral WR2721 can inhibit its own gastric emptying, we also determined the effect of intragastric administration of saline or WR2721 (5, 10, 20, 75 and 150 mg/kg) on gastric emptying of solids and liquids using radionuclide imaging as described above.

2. Role of the area postrema in the emetic and gastroplegic effects of WR2721 (12 beagle dogs).

We evaluated the role of the chemoreceptor trigger zone (area postrema), which is located on the floor of the fourth ventricle in WR2721-induced vomiting. These studies were performed in the dog because this animal more consistently vomits after administration of WR2721.

Before actual experiments were initiated, the dogs were habituated to the Pavlov stand during at least three 3-hour sessions.

a) Preoperative emetic drug tests

Apomorphine intravenously: (0.1 mg/kg); WR2721 intravenously (150 mg/kg)

b) Operative procedure

Ablation of the Area Postrema (AP) in chronic animals was carried under aseptic conditions. Dogs were anesthetized with sodium pentobarbital (40 mg/kg, IV), continued with 1% halothane in a 50/50 mix of nitrous oxide and oxygen. The trachea was intubated per os and the animal was placed under artificial ventilation. The neck was strongly ventroflexed with a headholder to gain good access to the foramen magnum. Exposure of the occipital bone was made by bilateral retraction of the dorsal neck muscle to provide a natural operative closure on completion of the procedure. The foramen magnum was enlarged nearly to the occipital ridge to permit upward retraction of the cerebellar nodulus for exposure of the medullary obex and floor of the fourth ventricle. The AP was destroyed by free-hand cauterization with a bent tip pencil type heat cautery with the aid of an operating microscope. Finally, the craniotomy was covered with gelfoam and the skin sutured with s.c. hidden stitch to avoid infection.

c) Postoperative care

No specific treatment was given postoperatively, although animals were observed daily during the postoperative period for temperature alterations, dehydration, and adequate food intake. The suture material placed on the skin was remove two weeks postsurgery.

d) Postoperative emetic drug tests

Same as preoperative tests after minimum convalescence of three weeks.

e) Observations

At the time of each study, gastric emptying of solids and liquids was measured using radionuclide imaging. In brief, after an overnight fast, the dog was fed 0.5 mCi Tc-99m-SC in vivo labeled raw chicken liver (20 gr) mixed with beef dog food (100 gr) and 0.1 mCi In-111-DTPA in 2 ml of water. Animals were then placed in a Pavlov stand for three hours. Objective quantitation of vomiting was possible as radiolabeled vomitus was detected. During the three hours of the study of gastric emptying in front of the gamma cameras, each dog was kept under continuous direct observation. Radionuclide imaging was performed at 10-min intervals for three hours after this injection, thus permitting the determination of the amount of Tc-99m and In-111 present in the stomach and in the intestine. One-min imagings were taken from the left and from the right of the animal, thus permitting a calculation of the geometric mean of the amounts of radioactivity measured in these 2 pictures. The percentage of solids (Tc-99m) and liquids (¹¹¹In) remaining in the stomach at each time interval was calculated as the ratio of the stomach contents divided by the sum of the gastric and intestinal contents.

f) Animal termination

After the postoperative studies were completed, each dog was deeply anesthetized with pentobarbital (50 mg/kg, IV). The brain was perfused-fixed in situ with formalin delivered through the ascending aorta. The hindbrain was then removed and stored in fixative (4% formaldehyde in DW).

g) Lesion histology

All brain specimens were sent to Dartmouth Medical School for histological processing and morphological analysis to match the work done by McCarthy, Borison and Douple in cats (Proc 33d Meeting of the Radiat Res Soc, 1985; Hh14;108A).

h) Analysis of the data.

Computer analysis of gastric motility and of gastric emptying permitted a quantitative analysis of gastric function. In addition, it allowed the study of the correlation between gastric motility and gastric emptying. Finally, the movements of the the animals during vomiting were recorded in an objective manner.

The statistical significance of differences observed for each measurement of gastric function (i.e., fractional emptying rate, acid output, etc...) was evaluated using a three-factor (treatment, time, and monkey) analysis of variance with repeated measures on the last two factors, the program LDU-040 (K.L. Dorn), and an IBM 370 computer (Division of Computer Research and Technology, National Institutes of Health, Bethesda, MD).

RESULTS

1. Emetic and gastroplegic effects of WR2721 in monkeys; involvement of opioids.

Vomiting was consistently observed in one monkey weighing 6.8 kg after administration of 150 mg/kg, and 75 mg/kg, but not after 75mg/kg, of slow i.v. injection of WR2721 performed over 10 min. In contrast, no vomiting was noted in any of the

other, smaller (2.5-5.0 kg), monkeys.

Gastric emptying of both liquids and solids was abolished for up to 60 min after administration of 150 mg/kg, 75 mg/kg, and 37.5 mg/kg of WR2721, i.v. (figs. 1 to 4). A similar dose dependent suppression of gastric emptying was observed after intragastric WR2721: the calculated ED_{50} was 7.3 ± 3.1 mg/kg for liquids and 8.8 mg/kg for solids.

Plasma β -endorphin was 7.6 ± 2.4 fmol/ml basally and these levels were not significantly modified by either saline, a mixed liquid/solid meal, or WR2721. Furthermore, naloxone did not prevent the WR2721-induced suppression of gastric emptying.

Gastric motor activity as measured by serosal strain gauges was markedly decreased during intravenous injection of 150 and 75 mg/kg WR2721 (figs. 5 and 6). The inhibition started within 2 min of the start of the injection and lasted for more than 60 min, suggesting that this effect is immediate and may be produced by one fifth of the total dose, i.e. as little as 15 mg/kg. Computer analysis of the recording demonstrated that this inhibition lasted for at least 2 hours, and that the stimulation induced by a meal given after WR2721 was markedly suppressed (fig. 7 and 8). The recordings of cutaneous electrogastragrams are currently being examined and analyzed using the computer programs developed for the analysis of tracing obtained from implanted strain gauges and electrodes.

2. Role of the area postrema in the emetic and gastroplegic effects of WR2721

During saline infusion, postremectomy (fig. 9) significantly increased gastric fractional emptying of solids and liquids compared to sham operation (fig 10). WR2721 produced vomiting in all sham operated animals, thereby preventing evaluation of gastric

emptying. WR2721 did not produce vomiting in any of the postremectomized dogs, although WR2721 slowed significantly gastric emptying of liquids, but not of solids (fig 11).

DISCUSSION

The present results demonstrate that WR2721 given either orally or as a slow intravenous infusion of the material diluted immediately before administration does not consistently produce vomiting in monkeys. In contrast, WR2721 suppressed gastric emptying in all 8 monkeys. Furthermore, suppression of gastric emptying of liquids and solids was maximum for all doses greater than 37.5 mg/kg, i.e. one fourth of the radioprotective dose. Thus, gastric emptying of solids and liquids is suppressed by oral and i.v. WR2721 with an ED₅₀ equal to 5 % of the radioprotective dose, and this action does not appear to be related to a release of endogenous opiates or to an activation of opiate receptors. This observation is relevant, in that a slowing of emptying probably allows the intragastric degradation of the compound before it can reach the small intestine; and thereby prevent its absorption after oral administration.

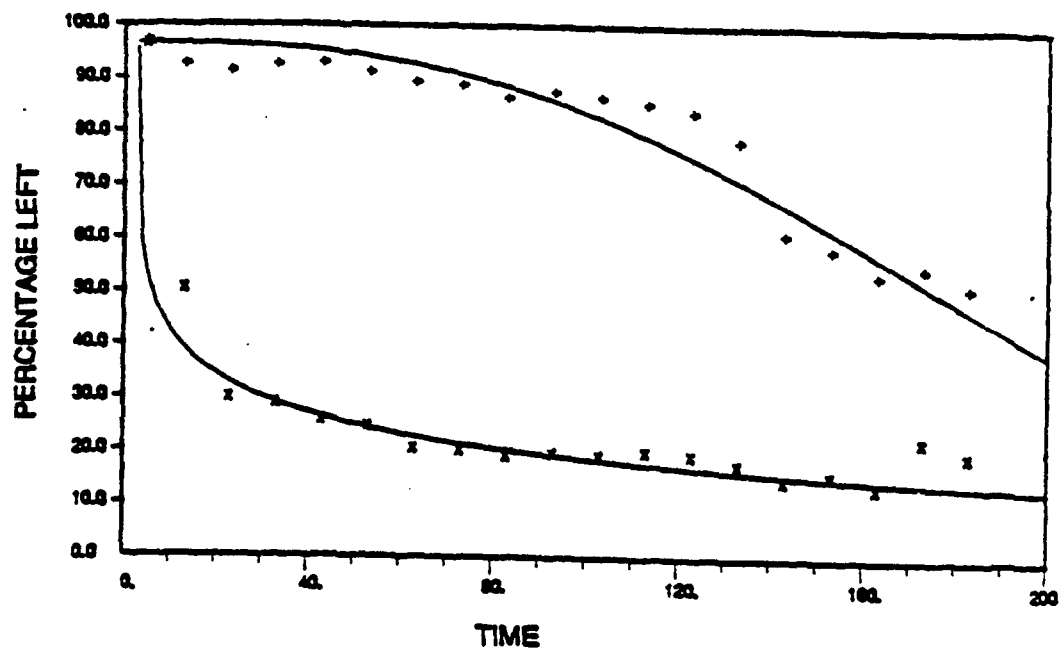
In order to better understand the mechanism of the suppression of gastric emptying, we have evaluated the effect of WR2721 on gastric motility. We found gastric mechanical activity was suppressed after one third of the total dose had been administered, suggesting that this effect occurred with a dose much lower than that needed for radioprotection (fig. 5). However, this phenomenon appears much more complex than previously anticipated and the computer analysis of the tracings required extensive software and hardware development (fig. 6 to 8). The method currently used

for the analysis of the tracings appears satisfactory, but additional work will be necessary to completely understand the alterations of the gastric peristalsis induced by WR2721.

The studies in dogs with postremectomies and sham operation suggest that the area postrema exerts an inhibitory role on gastric emptying. In addition, the slowing effect of WR2721 on gastric emptying of solids appears to be mediated by the area postrema since it is abolished in postremectomised dogs. Finally, the slowing effect of WR2721 on gastric emptying of liquids does not seem to depend on the emetic effect of this drug, or on an intact area postrema.

In the future, it is hoped that these findings will permit the development of strategies to treat and to prevent WR2721-induced vomiting and suppression of gastrointestinal transit.

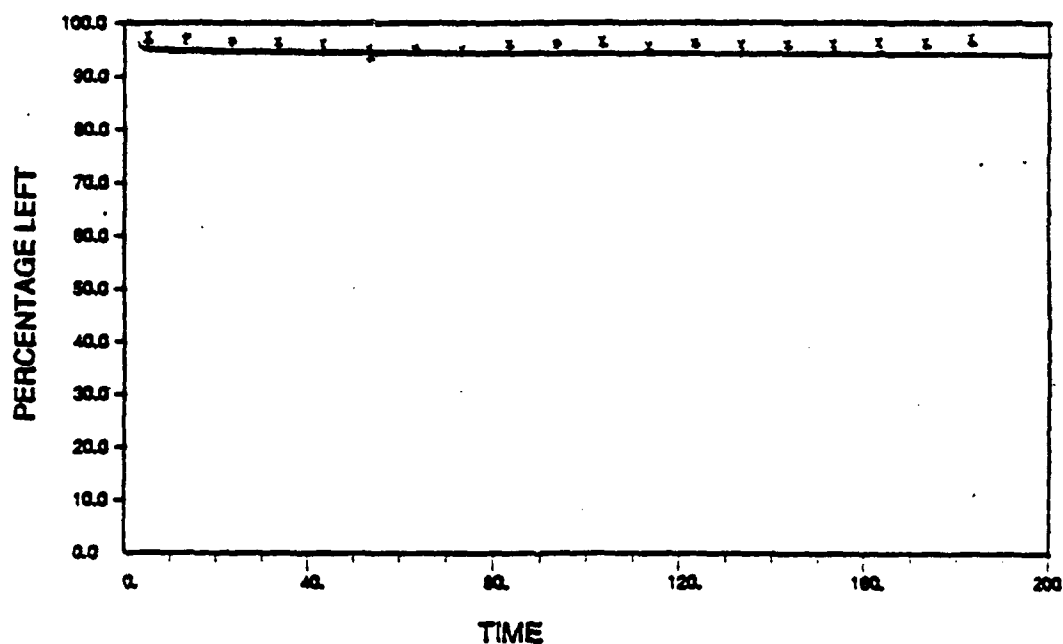
Fig 1: Percentage of the meal remaining in the stomach for solids (+) and liquids (x) over time in min, after injection of saline in one monkey. The parameters listed under the graph represent the initial and final estimates for half life (H) and early emptying (S) for solids and liquids, using a locally developed curve fitting computer program based on a power exponential.



	Liquid	Solid
Initial H	0.5473811E+02	0.5576171E+02
Initial S	0.1000000E+01	0.1000000E+01
Iterations 100	5	10
Final H	0.8948879E+01	0.1804215E+03
Final S	0.2891687E+00	0.2832385E+01
Sum of Least Squares	0.1288007E+02	0.2138781E+02

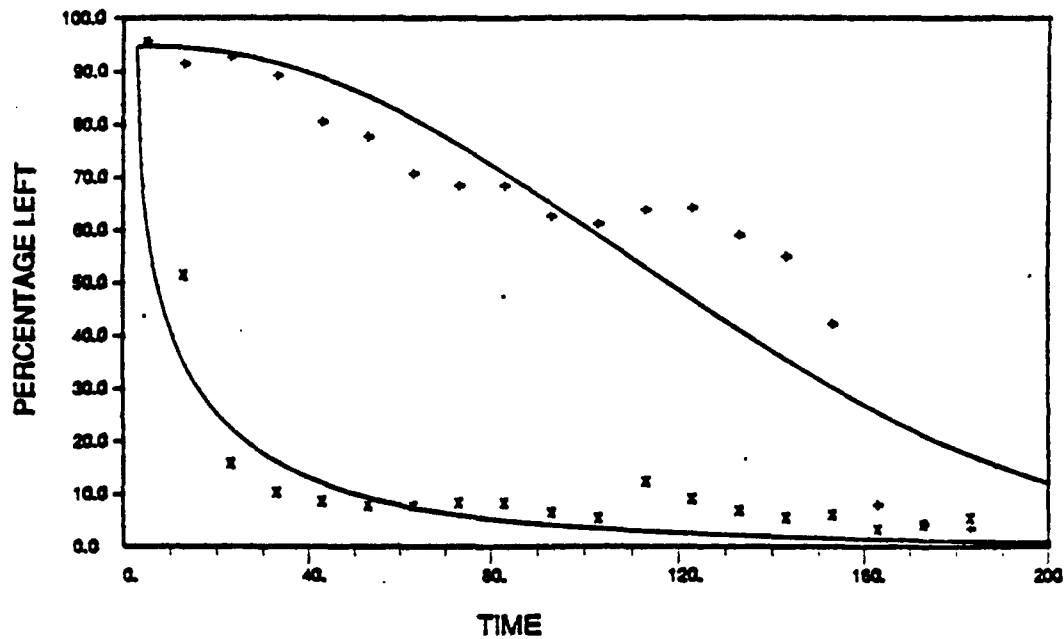
Fig 2:

Percentage of the meal remaining in the stomach for solids (+) and liquids (x) over time in min, after injection of WR2721 (150 mg/kg) in the monkey whose baseline data are shown in fig 1. This animal vomited after administration of the drug but before administration of the meal. The parameters listed under the graph represent the initial and final estimates for half life (H) and early emptying (S) for solids and liquids, using a locally developed curve fitting computer program based on a power exponential.



	Liquid	Solid
Initial H	0.1025056E+03	0.1025695E+03
Initial S	0.1000000E+01	0.1000000E+01
Iterations 100	100	13
Final H	0.2920797E+18	0.2027915E+08
Final S	0.9123939E-01	0.3246587E+00
Sum of Least Squares	0.1244974E+01	0.9134141E+00

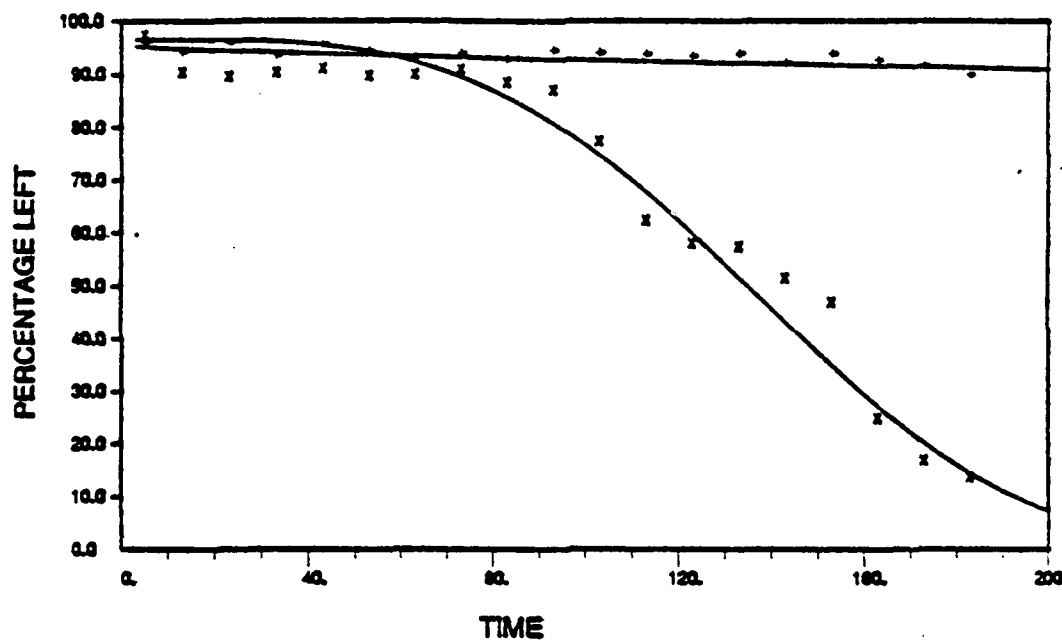
Fig 3: Percentage of the meal remaining in the stomach for solids (+) and liquids (x) over time in min, after injection of saline in another monkey. The parameters listed under the graph represent the initial and final estimates for half life (H) and early emptying (S) for solids and liquids, using a locally developed curve fitting computer program based on a power exponential.



	Liquid	Solid
Initial H	0.1870956E+02	0.6020548E+02
Initial S	0.1000000E+01	0.1000000E+01
Iterations 100	8	8
Final H	0.7935016E+01	0.1220529E+03
Final S	0.5228131E+00	0.2170182E+01
Sum of Least Squares	0.2990576E+02	0.1493580E+03

Fig 4:

Percentage of the meal remaining in the stomach for solids (+) and liquids (x) over time in min, after injection of WR2721 (150 mg/kg) in the monkey whose baseline data are shown in fig 3. This animal did not vomit after administration of the drug. The parameters listed under the graph represent the initial and final estimates for half life (H) and early emptying (S) for solids and liquids, using a locally developed curve fitting computer program based on a power exponential.



	Liquid	Solid
Initial H	0.5061116E+02	0.9657872E+02
Initial S	0.1000000E+01	0.1000000E+01
Iterations 100	8	14
Final H	0.1368178E+03	0.8955831E+04
Final S	0.3400052E+01	0.7053912E+00
Sum of Least Squares	0.3103495E+02	0.9997029E+00

Fig 5: Recording of tracings obtained in one monkey during the slow intravenous infusion of WR2721 (150 mg/kg). T₁: proximal stomach; T₂: mid-stomach; T₃: distal stomach. Note that the motility of the proximal stomach is much weaker than that of the mid- and distal stomach; therefore the dramatic effect seen in T₂ and T₃ (and further illustrated in figs. 7 and 8) is not visible in T₁, although it can be detected using the computer program as illustrated in fig. 6. Values listed under the tracings present the quantitative evaluation of three parameters for T₂.

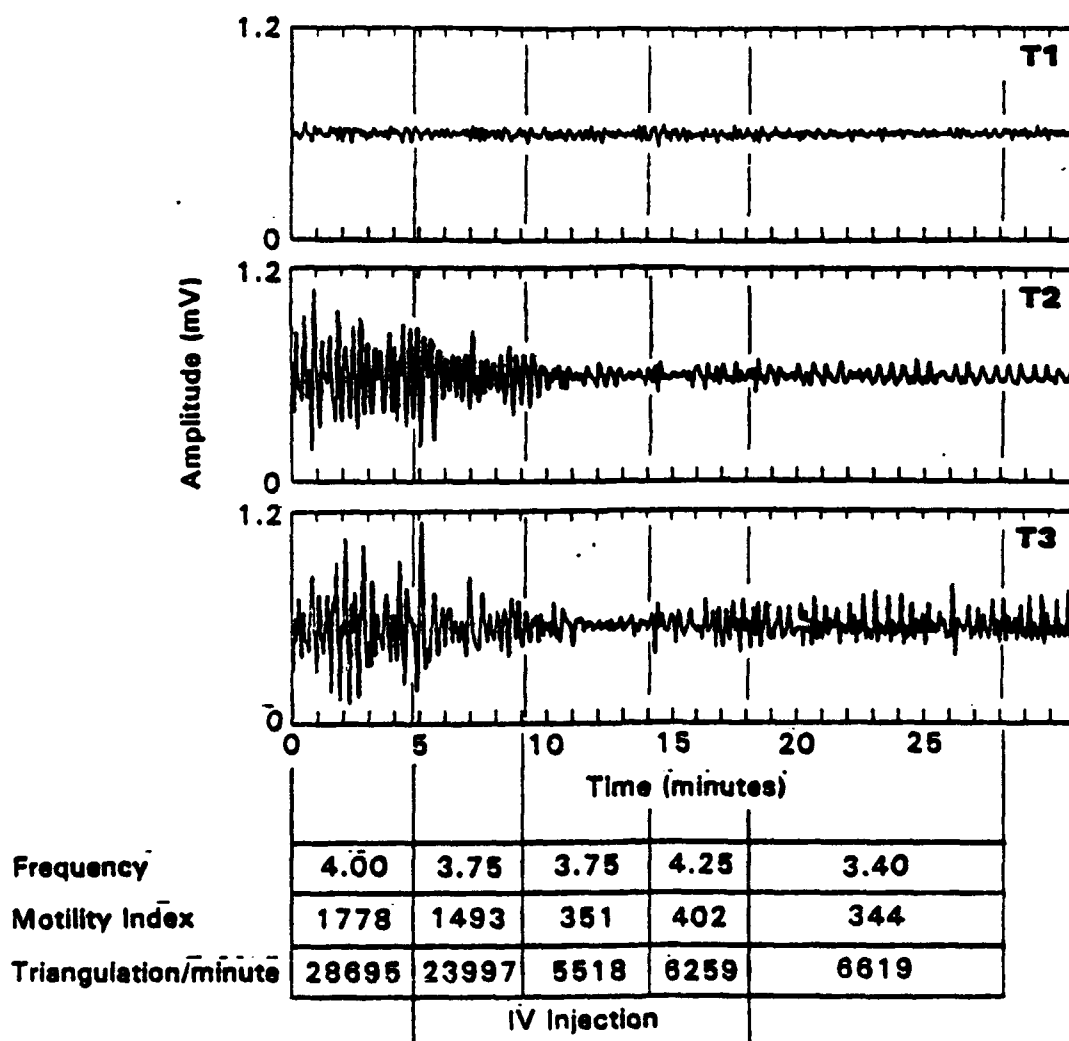


Fig 6:

Graphic representation of the parameters calculated from the tracing of the proximal stomach which is illustrated in fig 5 (T_1). Locally developed programs were used for these calculations. Note that the meal given at 30 min produces no significant changes of the parameters, and that the parameters representing motor activity (amplitude, motility index and area under the curve), but not the frequency, are decreased after WR2721 (given at 120 min). A second meal, given after WR2721 is emptied much more slowly (see figs. 2 and 3), and produces no change in frequency, but a stimulation of the activity parameters, which, however, remain lower than before WR2721.

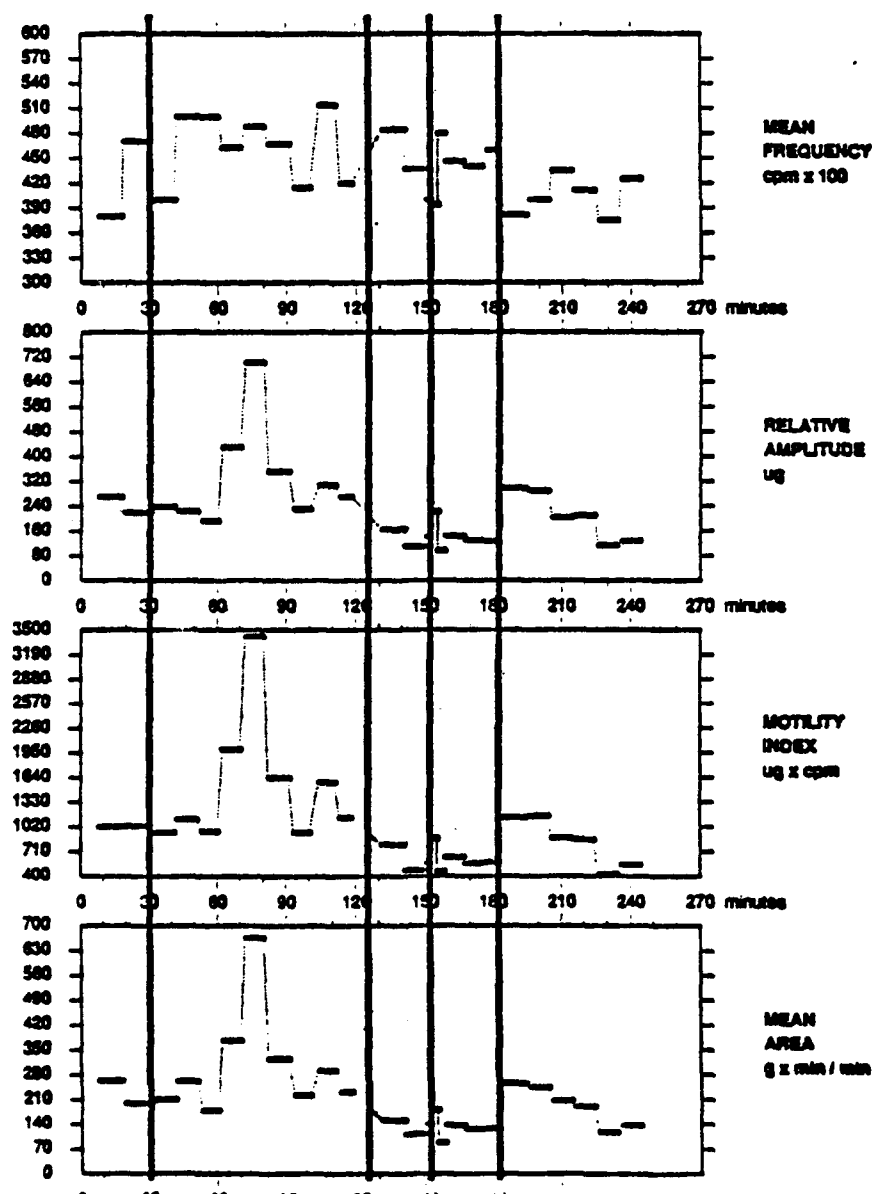


Fig 7:

Graphic representation of the parameters calculated from the tracing of the mid-stomach which is illustrated in fig 5 ($T_{1/2}$). Locally developed programs were used for these calculations. Note that the meal given at 30 min produces an increase of the parameters representing motor activity (amplitude, motility index and area under the curve), but not the frequency, and that all the parameters are decreased after WR2721 (given at 120 min). A second meal, given after WR2721 at 185 min is emptied much more slowly (see figs. 2 and 3), and produces no change in frequency, but a weak stimulation of the activity parameters, which, however, remain lower than before WR2721.

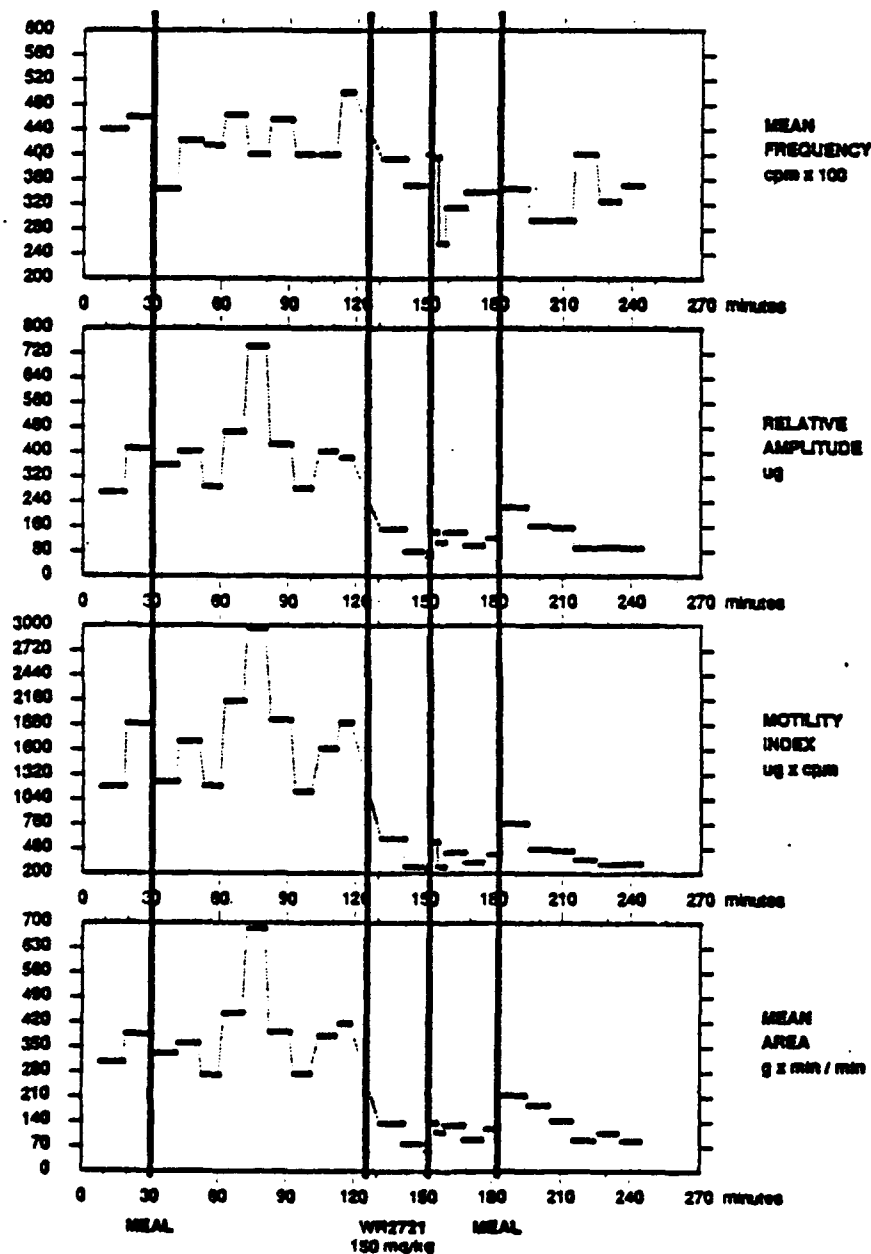


Fig 8:

Graphic representation of the parameters calculated from the tracing of the mid-stomach which is illustrated in fig 5 ($T_{1/2}$). Locally developed programs were used for these calculations. Note that the meal given at 30 min produces an increase of the parameters representing motor activity (amplitude, motility index and area under the curve), but not the frequency, and that all the parameters are decreased after WR2721 (given at 120 min). A second meal, given after WR2721 at 185 min is emptied much more slowly (see figs. 2 and 3), and produces no change in frequency, but a weak stimulation of the activity parameters, which, however, remain lower than before WR2721.

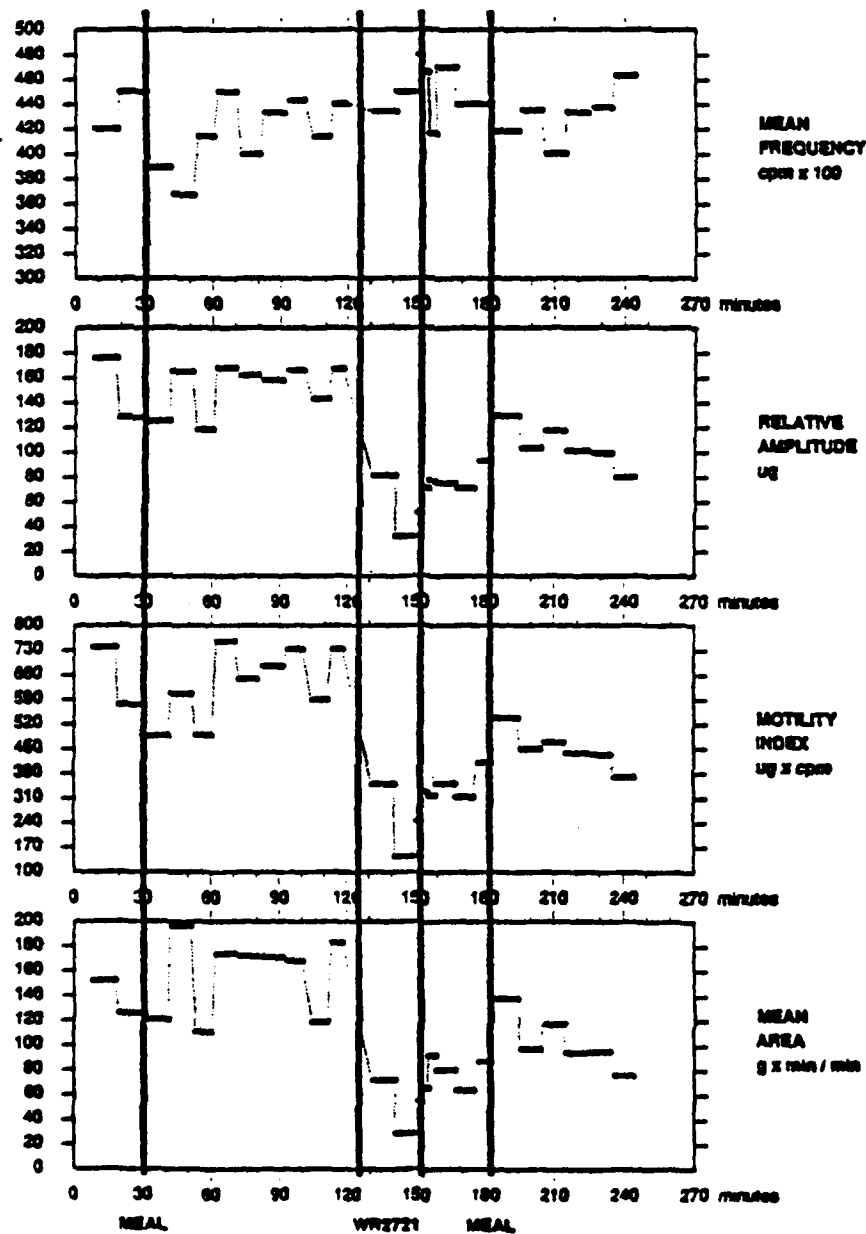


Fig 9: Histological illustration of the effect of surgical interventions on the medulla in dogs 3 weeks after surgery. On the left, sham operated animals, showing intact area postrema. On the right, the area postrema has been removed but other nuclei are intact.

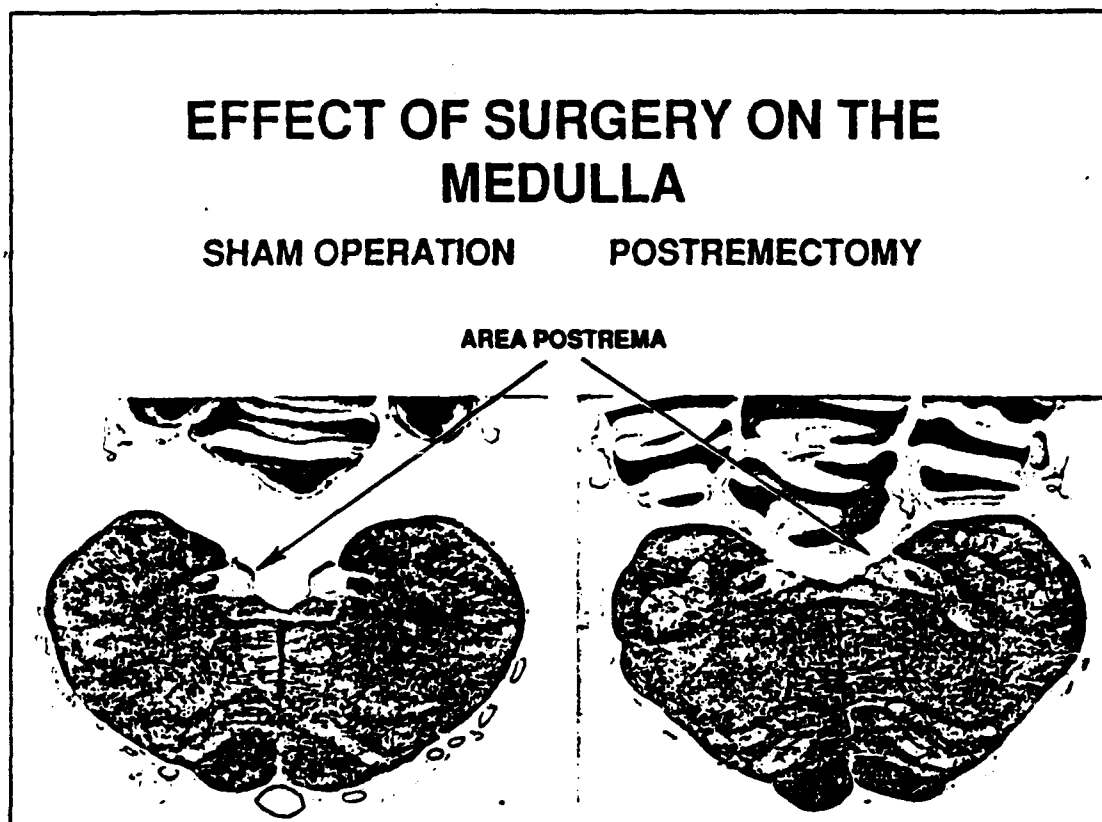


Fig 10: Effect of postremectomy on fractional gastric emptying rate of solids (K_s) and liquids (K_L) in %/hour. Postremectomy significantly increased K_L and K_s (*: $P < 0.05$).

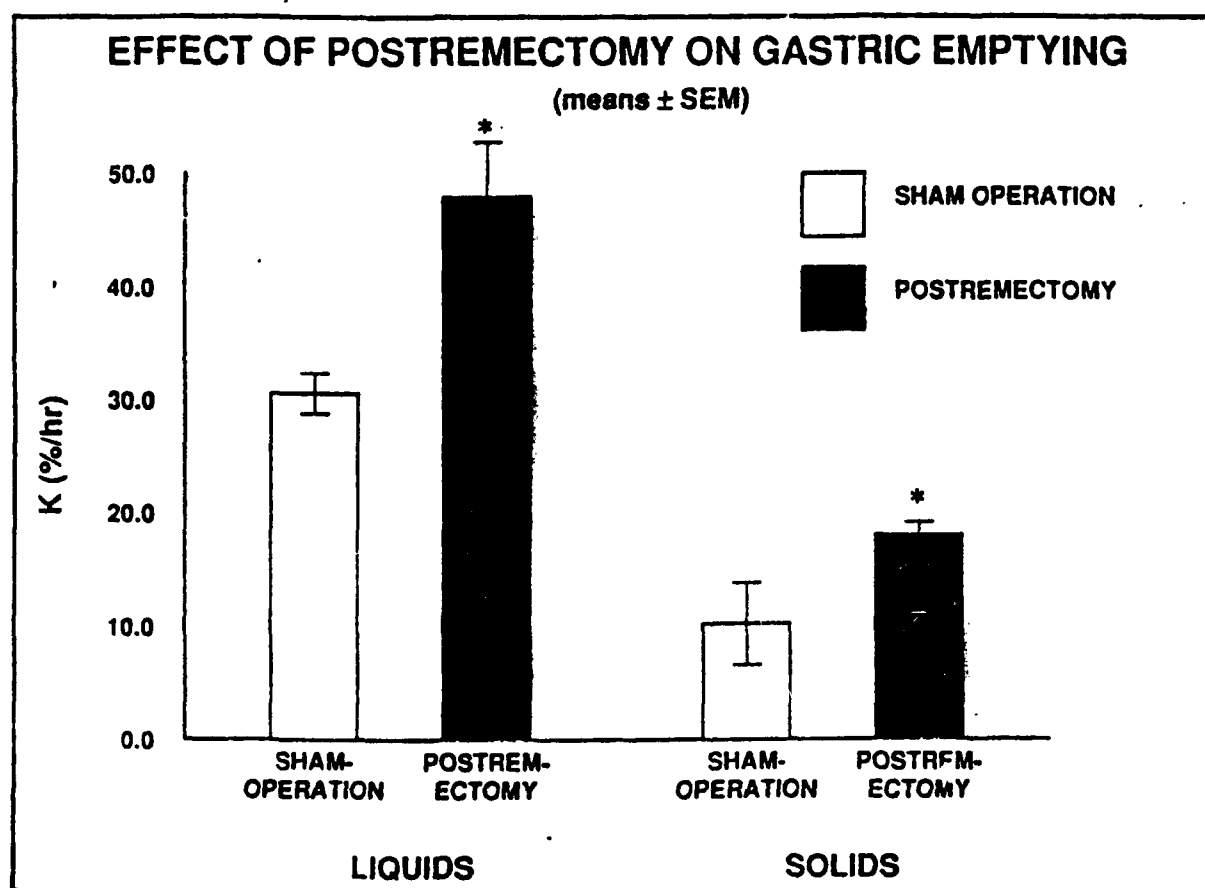


Fig 11: Effect of WR2721 on fractional gastric emptying rate of solids (K_s) and liquids (K_L), in %/hour, in postremectomized dogs, i.e. those animals in which WR2721 did not produce vomiting. In this animal model, WR2721 significantly suppressed K_L (\ddagger : $P < 0.05$) but not K_s .

